

CLAIMS:

1. An isolated tumor necrosis receptor associated factor (TRAF) capable of specific association with the intracellular domain of a native type 2 TNF receptor (TNF-R2).

2. The TRAF of claim 1 that is murine.

3. The TRAF of claim 1 that is capable of specific association with the intracellular domain of a native human TNF-R2.

4. The TRAF of claim 1 that is capable of specific binding to the intracellular domain of a native human TNF-R2.

5. The TRAF of claim 1 that is native.

6. The TRAF of claim 5 in homodimeric form.

7. The TRAF of claim 5 associated with another TRAF to form a heterodimer.

8. The TRAF of claim 5 that is TRAF1 (SEQ. ID. NO: 2) or TRAF2 (SEQ. ID. NO: 4).

9. The TRAF of claim 1 which comprises a domain having at least about 50% sequence identity with the aa272-501 amino acid region of the TRAF2 amino acid sequence (SEQ. ID. NO: 4).

10. The TRAF of claim 1 encoded by nucleic acid molecule capable of hybridizing, under stringent conditions, to the complement of the nucleotide sequence encoding amino acids 272-501 of the TRAF2 amino acid sequence (SEQ. ID. NO: 4).

5 SubC1
11. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a TRAF of claim 1.

2 12. A vector comprising the nucleic acid molecule of claim 11 operably linked to control sequences recognized by a host cell transformed with the vector.

3 13. A host cell transformed with a vector of claim 12.

15 14. A molecule capable of disrupting the interaction of a TRAF and a native TNF-R2.

20 15. An antibody capable of specific binding to a native TRAF polypeptide.

16. A hybridoma cell line producing an antibody of claim 15.

B
25 Sub-C2
17. A method of using a nucleic acid molecule encoding a TRAF_n comprising expressing such nucleic acid molecule in a cultured host cell transformed with a vector comprising such nucleic acid molecule operably linked to control sequences recognized by said host cell, and recovering the polypeptide encoded by said nucleic acid molecule from the host cell. according to claim 11

18. A method for producing a TRAF polypeptide comprising inserting into the DNA of a cell containing nucleic acid encoding said polypeptide a transcription modulatory element in sufficient proximity and orientation to the nucleic acid molecule to influence the transcription thereof.

19. A method of determining the presence of a TRAF polypeptide comprising hybridizing DNA encoding such polypeptide to a test sample nucleic acid and determining the presence of TRAF polypeptide DNA.

20. An isolated nucleic acid molecule encoding a fusion of an intracellular domain sequence of a native TNF-R2 to the DNA-binding domain of a transcriptional activator.

21. The nucleic acid molecule of claim 20, wherein said transcriptional activator is yeast GAL4.

22. An isolated nucleic acid molecule encoding a fusion of a TRAF to the activation domain of a transcriptional activator.

23. The nucleic acid molecule of claim 22, wherein said transcriptional activator is yeast GAL4.

24. A vector comprising the nucleic acid molecule of claim 20.

25. A vector comprising the nucleic acid molecule of claim 22.

26. An assay for identifying a factor capable of specific binding to the intracellular domain of a native TNF-R2, comprising

(a) expressing nucleic acid molecules encoding a polypeptide comprising a fusion of an intracellular domain sequence of a native TNF-R2 to the DNA-binding domain of a transcriptional activator, and a fusion of a candidate polypeptide factor to the activation domain of a transcriptional activator, in a single host cell carrying a reporter gene;

(b) monitoring the binding of said candidate factor to the intracellular domain of TNF-R2 by detecting a signal of the molecule encoded by said reporter gene.

27. As assay for identifying a factor capable of specific association with the intracellular domain of a native TNF-R2, comprising

(a) expressing nucleic acid molecules encoding a polypeptide comprising a fusion of an intracellular domain sequence of a native TNF-R2 to the DNA-binding domain of a transcriptional activator, and a fusion of a candidate factor to the activation domain of a transcriptional activator, in a single host cell transfected with nucleic acid encoding a polypeptide factor capable of specific binding to said TNF-R2, and with nucleic acid encoding a reporter gene; and

(b) monitoring the association of said candidate factor with said TNF-R2 or with said polypeptide factor capable of specific binding to said TNF-R2 by detecting the signal of the polypeptide encoded by said reporter gene.

28. A method of amplifying a nucleic acid test sample, comprising priming a nucleic acid polymerase reaction with nucleic acid encoding a TRAF polypeptide capable of specific association with the intracellular domain of a native TNF-R2.

29. A method for detecting a nucleic acid sequence coding for a polypeptide molecule which comprises all or part of a TRAF polypeptide or a related nucleic acid sequence, comprising contacting the nucleic acid sequence with a detectable marker which binds specifically to at least part of said nucleic acid sequence, and detecting the marker so bound.

30. A method for the prevention or treatment of a pathological condition associated with a TNF biological activity mediated, fully or partially, by a TNF-R2, comprising administering to a patient in need a preventatively or therapeutically effective amount of a TRAF or a molecule capable of disrupting the interaction of a TRAF and said TNF-R2.

31. An assay for identifying a molecule capable of disrupting the association of a TRAF with the intracellular domain of a native TNF-R2, comprising contacting a cell coexpressing a native TNF-R2, a native TRAF polypeptide and a reporter gene with a candidate molecule, and monitoring the ability of said candidate molecule to disrupt the association of said TRAF and TNF-R2 intracellular domain sequence by detecting the molecule encoded by the reporter gene.

32. As assay for identifying a molecule capable of disrupting the association of a TRAF with the intracellular domain of a native TNF-R2, comprising contacting a cell expressing 1. a fusion of an intracellular domain sequence of a native TNF-R2 to the DNA-binding domain of a transcriptional activator, 2. a fusion of a native TRAF polypeptide to the activation domain of said transcriptional activator, and 3. a reporter gene, with a candidate molecule, and monitoring the ability of said candidate molecule to disrupt the

association of said TRAF and TNF-R2 intracellular domain sequence by detecting the molecule encoded by the reporter gene.

5 33. An assay for identifying an inhibitor of the interaction of a TRAF protein with CD40 comprising contacting recombinant host cells coexpressing a TRAF protein capable of direct or indirect binding of CD40, CD40 and a reporter gene the expression of which is dependent on the CD40:TRAF interaction, with candidate inhibitors and selecting a molecule which inhibits the expression of said reporter gene.

10 34. The assay of claim 33 wherein said TRAF protein is TRAF2.

15 35. The assay of claim 33 wherein said recombinant host cells coexpress TRAF1 and TRAF2.

36. The assay of claim 33 wherein said reporter gene is NF- κ B dependent.

20 37. The assay of claim 36 wherein said NF- κ B dependent reporter gene is an E-selectin-luciferase reporter gene construct.

38. The assay of claim 33 which is performed in the two-hybrid format.

25 39. An assay for identifying an inhibitor of the interaction of a TRAF protein with LMP1 comprising contacting recombinant host cells coexpressing a TRAF protein capable of direct or indirect binding of LMP1, LMP1 and a reporter gene the expression of which is dependent on the LMP1:TRAF

interaction, with candidate inhibitors and selecting a molecule which inhibits the expression of said reporter gene.

40. The assay of claim 39 wherein said TRAF protein is TRAF2.

41. The assay of claim 39 wherein said recombinant host cells coexpress TRAF1 and TRAF2.

42. The assay of claim 39 wherein said reporter gene is NF- κ B dependent.

43. The assay of claim 42 wherein said NF- κ B dependent reporter gene is an E-selectin-luciferase reporter gene construct.

44. The assay of claim 39 which is performed in the two-hybrid format.

45. TRAF2(87-501) or a functional derivative thereof capable of inhibiting a biological activity mediated by TNF-R2, CD40 or LMP1.

46. The functional derivative of claim 45 which is capable of inhibiting TRN-R2-, CD4- or LMP1-mediated NF- κ B activation.

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a'

add
b³